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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>5</sup> :</b> <b>C12N 11/14, 9/98 // C11D 3/386</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 94/26883</b> <b>(43) International Publication Date:</b> 24 November 1994 (24.11.94)
<b>(21) International Application Number:</b> PCT/EP94/01642 <b>(22) International Filing Date:</b> 18 May 1994 (18.05.94)  <b>(30) Priority Data:</b> 93201428.5      18 May 1993 (18.05.93)      NL  <b>(71) Applicant (for all designated States except US):</b> GIST-BROCADES N.V. [NL/NL]; Wateringseweg 1, P.O. Box 1, NL-2600 MA Delft (NL).  <b>(72) Inventors; and</b> <b>(/5) Inventors/Applicants (for US only):</b> ANDELA, Carl, Sidonius, Maria [NL/NL]; Grabijnhof 7, NL-2625 LL Delft (NL). FEIJEN, Jan [NL/NL]; Vleekade 36, NL-2725 AZ Zoetermeer (NL). DILLISSEN, Marc [BE/BE]; Antwerpsesteenweg 784, B-9000 St. Amandsberg (BE).  <b>(74) Agents:</b> HUYGENS, Arthur, Victor et al.; Gist-Brocades N.V., Patents and Trademarks Dept., Wateringseweg 1, P.O. Box 1, NL-2600 MA Delft (NL).		<b>(81) Designated States:</b> AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> PROCESS FOR DUST-FREE ENZYME MANUFACTURE  <b>(57) Abstract</b>  New enzyme granules are provided with improved properties. The granules are based on core particles having a good pore size and pore size distribution to allow an enzyme solution to enter into the particle. Accordingly, the core material comprises the enzyme in liquid form, thus eliminating the drawback of processing powdered enzymes. Still, good flowability and metering properties are obtained. The enzyme-containing granules are preferably coated with a film-forming macromolecular material. These granules have lower dustiness and improved mechanical strength. Also a method of preparing these enzyme granules is provided.		

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**PROCESS FOR DUST-FREE ENZYME MANUFACTURE****Field of the Invention**

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The present invention relates to novel enzyme granules and a process for the preparation thereof.

**Background of the Invention**

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Many commercially useful enzymes are produced by microorganisms such as bacteria, yeast and fungi. These enzymes are especially useful in detergents, starch and textile processing, and in feed and food applications.

15

Examples of enzymes useful in detergent application include proteases, amylases, lipases and cellulases. The enzymes are produced by fermentation and then recovered, purified and concentrated into a liquid or brought in dry form. Suitable recovery techniques include filtration, centrifug-

20

ation, membrane filtration, precipitation, crystallisation, chromatography, spray drying, etc.

Because of dermatologic and other health problems which might occur in connection with dry enzyme and in particular dry protease preparations, the dust levels in such preparations should be as low as possible. To achieve this the dry enzymes are usually brought in granule form, for which several granulation techniques have been developed. See, for example, U.S. Patents Nos. 4,009,076, 4,016,040, 4,078,368, 4,242,219, etc.

30

In order to further reduce the enzyme dust release and to protect the granules, most of the granules are coated after their production with a film-forming macromolecular material.

35

Basically two types of granules can be distinguished. In the first type the compound of interest is mixed through the granule and in the second type the compound of interest is located around the core of the granule. It will be clear that in the latter type the concentration of the compound of interest (e.g. an enzyme) at the surface of the core is very high as compared to a granule where the compound is mixed

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throughout the granule. Consequently, when the coating is inadequate or damaged, the compound will be exposed to the environment at a higher level than in case of a granule with an uniform distribution of the compound in question.

5 JP 58-179492A discloses the preparation of enzyme granules with a protective coating. Here a fluid bed dryer is used to first spray e.g. a liquid enzyme concentrate onto a core material and subsequently spray a coating material containing a cellulose derivative onto the granule, while  
10 drying takes place during the whole process.

EP-A-0193829 describes a method for the production of dust free enzyme containing particles by coating hydratable core particles with an enzyme and then with a film-forming material. Coating is carried out by suspending the core  
15 particles in a fluidized bed dryer, spraying an aqueous slurry of enzyme onto the core particles while suspended, and evaporating water to leave a dried enzyme coat on the particles. The resultant enzyme-coated particles, while still suspended in the fluidized bed, are sprayed with a solution or  
20 dispersion of the macromolecular material, and dried to remove solvent to leave a coating of the macromolecular material.

WO 90/09428 discloses a detergent additive granulate which comprises a core with a primary detergent additive, e.g. an enzyme, surrounded by a shell comprising a secondary  
25 detergent additive, e.g. another enzyme, a binder, and granulating agents, and optionally a filler, and a protective coating between the core and the shell, whereby the shell comprises cellulose or artificial fibres, and whereby the core optionally may also comprise cellulose or artificial fibres.  
30 The detergent additive granulate is said to exhibit a high physical strength, and the primary and secondary detergent additives are separated from each other and/or from harmful environmental factors.

WO 93/07263 discloses a granular enzyme composition  
35 which comprises a core, an enzyme layer and an outer coating layer. The enzyme layer, and optionally the core and coating layers, contain a vinyl polymer. The granular enzyme

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composition is said to have reduced tendencies to form dust and leave residue, and exhibit improved stability and delayed release characteristics. The method for making such enzyme-containing granules is said to have greatly reduced processing  
5 time.

The present invention provides new enzyme granules with improved properties. As compared to the prior art cited above the granules according to the present invention have the advantage that the compound of interest is not only located at  
10 the surface of the granule.

#### Summary of the Invention

According to the first aspect of the invention enzyme  
15 granules are provided comprising porous core material in which the enzyme in solution is adsorbed.

The enzyme-containing granules are preferably coated with a film-forming macromolecular material. Such granules have an improved mechanical strength.

20 The invention further provides an efficient method of preparing the novel enzyme granules.

#### Detailed Description and Preferred Embodiments

25 The granules according to the present invention are based on core particles having a good pore size and pore size distribution to allow an enzyme solution to enter into the particle. Accordingly, the core material comprises the enzyme in liquid form, thus eliminating the drawback of processing  
30 powdered enzymes.

The core materials available up till now do not have the required characteristics. The pore diameter should not be too big, since this will inter alia reduce the strength of the particle, and not too small, since this will prevent the  
35 compound of interest, e.g. an enzyme, entering into the pores.

European Patent Application EP-A-0542351 (published on May 19, 1993, i.e. after the priority date of the present

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patent application) discloses a process for the preparation of salt granulates. The products which are obtainable by this process are very suitable for use as core material in enzyme applications. The level of porosity is important with respect to the economics of the process.

In a further aspect of the invention a method is provided for efficiently preparing the new enzyme granules which comprises the following steps:

- 10 (a) preparing a non-aqueous enzyme solution
- (b) applying said enzyme solution onto porous core material whereby the enzyme solution is absorbed into the porous core material
- (c) drying the resulting enzyme granules, and,
- 15 optionally,
- (d) coating the enzyme granules with a macromolecular, film-forming material.

The enzyme solution may be prepared in various ways, 20 mainly depending on the physical characteristics of the enzyme used. Suitable solvents for the purpose of the invention include ethylene glycol, propylene glycol, liquid polyethylene glycols (PEG's) such as PEG 200 and PEG 400, and glycerol. In certain cases it may be useful to prepare first an aqueous 25 enzyme solution and to add the non-aqueous solvent. The water is then partly or entirely removed, for example by evaporation. The "non-aqueous" enzyme solution may contain a certain amount of water, for example 10-20%, but this should not have an adverse affect on the various components of the granulate, in 30 particular the porous core material. Instead of an enzyme solution also an enzyme slurry may be used in certain instances which will be clear to the man skilled in the art.

The absorption of enzyme into the particle can be done in various way. Both from an enzyme containing aqueous liquid 35 or slurry (e.g. a concentrated fermentation broth) as from an enzyme containing non-aqueous liquid or slurry (e.g. non ionics, alcohols etc.), or a combination thereof.

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Suitable porous core material includes soda, NaCl and silica and is commercially available. The porous core material is preferably prepared by the method described in EP-A-0542351.

The enzyme solution can be brought onto the porous  
5 core material in various ways which are all known in the art, for example using mixing devices or a fluidized bed or a mixer-fluid bed dryer combination. Process steps (b) and (c) may suitably be combined.

If desired, one or more protective coating layer(s)  
10 can be brought onto the core to yield a dust free enzyme containing particle. Suitable coating materials are frequently described in the literature, see e.g. JP 58-179492A and EP-A-0193829. They include cellulose coatings or cellulose based coatings containing hydroxypropylcellulose, methyl cellulose,  
15 hydroxypropyl methyl cellulose and/or hydroxyethyl cellulose. Also acrylic polymers like EUDRAGIT® (Röhm Pharma) can be applied. The amounts of coating to be applied may vary in fairly wide ranges but is usually between 0.1 and 25% by weight, for the cellulose based coatings preferably between 5  
20 and 25 wt%.

Coating layers can also be used to add other useful compounds to the granule. It is even possible to prepare multilayer granules wherein various coating layers have different functions, for example to stabilize the compounds  
25 which are present, to add colour etc.

In certain applications which will be clear to the skilled person porous core material is suitably used which dissolves slowly or which liberates the absorbed enzyme slowly. This technology in combination with coating technology permits  
30 a composition of a granule where several compounds can be released in a sequence according to demand. An example hereof is a granule where a protease is absorbed into a porous core, a lipase is coated on the core and finally a coating layer is brought onto the granule.

35 The entire process for preparing the enzyme granules according to the invention (absorption, coating with coating material and other compounds) may be suitably carried out in



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one apparatus, for example a mixer or a fluidized bed.

The process according to the invention has several advantages. When the process is performed in more than one step, the (enzyme) dust formation is reduced during transport of the particles between the apparatus. Besides, the absorption of an enzyme solution or slurry in a porous particle is much faster than e.g. spraying an enzyme solution or slurry onto a core in a fluid bed or than mixing an enzyme with a non-porous carrier.

With the present porous material it is also possible to obtain a sequential or controlled release by means of other technologies than adjusting the composing or location of the coating layer(s).

The following examples are offered by way of illustration and not by way of limitation.

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EXPERIMENTAL

The protease used in the examples is the high alkaline protease PB92, see U.S. Patent No. Re 30,602, which is  
5 commercially available from Gist-brocades N.V. under the trade mark MAXACAL®.

The lipase used in the examples is the lipase which is obtainable from the strain Pseudomonas pseudoalcaligenes M-1 (CBS 473.85), see e.g. EP-B-0218272, and U.S. Patents Nos.  
10 4,933,287 and 5,153,135.

The chymosin used in the examples is produced by a transformed yeast strain of Kluyveromyces lactis and commercially available from Gist-brocades N.V. under the trade mark MAXIREN®. See e.g. EP-B-0096430 and EP-B-0301669 and U.S.  
15 Patent No. 4,859,596.

Example 1

Soda cores with a porosity of 6 (Soda ash Dense®), 16  
20 (Soda ash Compact®) and 38% (Soda ash Sorbent®), respectively, were obtained from Akzo N.V. (see also EP-A-0542351). The soda was sieved to obtain a particle size between 315-710 µm.

Enzyme liquids were produced by adding ethylene glycol and propylene glycol, respectively, to a concentrated aqueous  
25 protease solution. The water content of the liquid was then reduced to approximately 10% by evaporation. The protease concentration in the liquid was 3.7 MADU/g (=  $3.7 \times 10^6$  ADU/g). The liquid was then sprayed onto the soda particles. The particles were completely filled with the liquid up to the  
30 porosity level defined by the particles.

Spraying and absorption were carried out in a rotating vessel (enzyme liquid inlet temperature 30-50°C). The material was then coated in a fluid bed dryer essentially according to the method described in JP 58-179492A or EP-A-0193829) and dust  
35 free enzyme granules were obtained.

The non-aqueous protease solutions with ethylene glycol, propylene glycol, PEG 400 and glycerol were produced

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with a protease activity varying from 3.4 to 3.7 MADU/g. The liquid absorption process resulted in an enzyme yield of 61% to 98% based on enzyme activity, depending on the water content of the non-aqueous solution. The tests were carried out with soda cores of 6%, 16 and 38% porosity (see above).

After addition of the protease containing liquid the particles were coated in a fluidized bed coater (inlet air temperature 65°C, outlet temperature 40°C). Good mass yield was obtained with high retention of enzymatic activity.

#### EXAMPLE 2

Protease granules were prepared in the same way as described in Example 1 but using porous NaCl cores instead of soda cores. These NaCl cores were prepared essentially according to the method described in EP-A-0542351 and had a porosity of 15%. These cores were filled with a non-aqueous liquid containing a protease with an activity of 3.4 MADU/g. This resulted in a granulate which had an activity of 440.000 ADU/g (enzyme yield >97%). After coating in a fluidized bed with a cellulose based coating (20% w/w) under the same conditions as mentioned above, coated granules were obtained which had an activity of 397.500 ADU/g. Again a good mass yield was obtained (>92%).

#### EXAMPLE 3

A similar experiment was carried out with chymosin. Chymosin was dissolved in propylene glycol. The liquid absorbed well in the porous NaCl, which may absorb 15 % of its weight. This resulted in the same good enzyme yields and loading of the porous material.

#### Example 4

Porous core material (Soda ash Compact , 600 g) with a particle size of 300-710 µm was introduced in a fluid bed

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dryer. A solution of ethylene glycol containing a lipase and a non-ionic (Triton X114®) was introduced into the fluid bed dryer at a temperature of 38°C and sprayed onto the porous particles. The water was evaporated and the non-ionic and the lipase were absorbed into the particles. A coating layer was then sprayed onto the particles and dust-free granules were obtained.

#### EXAMPLE 5

10

In a similar way as described in Example 4 protease granules were made. The protease containing solutions of ethylene glycol and propylene glycol, respectively, were sprayed into the fluid bed directly on top of the porous material, which was fluidized (particle size between 400 and 600  $\mu\text{m}$ ). Both when the protease was dissolved in ethylene glycol and propylene glycol, the liquid was absorbed well into the material.

After applying an additional cellulose based coating (SEPIFILM® of Seppic) in the fluidized bed coater, and sieving over 300 and 600  $\mu\text{m}$  sieves, the dust levels were further reduced.

Four samples of protease granules which were prepared in a similar way as described above and coated with cellulose based coating material (20% w/w), were tested in the Heubach attrition test on dust formation (see WO 93/07263). In this test, enzyme dust levels below 0.5 mg/20 g are considered extremely low. Total dust figures below 10 mg/20 g are equally considered extremely low. The results are given in Table 1.

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Table 1Heubach attrition levels of 4 coated protease granulate samples

5		Heubach attrition levels			
	Sample #	1	2	3	4
	Enzyme dust [mg/20 g of sample, based on 300.000 ADU/g]	0.45	0.31	0.14	0.20
10	Total dust [mg/20 g of sample]	7.3	5.6	7.6	5.9

It appeared that the uncoated granules were smeared under the test conditions. In contrast, the coated particles  
15 apart from their lower dust level were strong enough to withstand the crushing forces of the steel balls of the Heubach attrition test.

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## Example 6

Various porous core materials (soda, NaCl and silica) were filled with non-aqueous protease solutions. Ethylene glycol and polyethylene glycol were used as the solvents.

Soda ash Sorbent® (see Example 1) is able to absorb a liquid upto 37% by weight (based on the soda), the NaCl used may absorb 15% of its weight, while the silica may absorb 100%.

Table 2 shows the residual enzyme activity after absorption of various enzyme containing liquids into various core materials.

Table 2

Protease yields measured after partial and complete loading of various porous materials

	product	wt% of liquid absorbed							
		5	10	15	20	25	30	37	100
residual enzyme activity [%]	Soda ash Sorbent®	100	102	90	93	93	92	94	
	NaCl	95	101	97					
	silica								102

## Example 7

The obtained material was tested for its protease stability at 7°C (at ambient relative humidity) using various non-aqueous liquids. Table 3 shows the results of the stability tests of the protease which is absorbed into the soda.

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Table 3Stability of the absorbed protease in the time

5	liquid in which the protease is dissolved <sup>*)</sup>	storage time (months)		
		0	2	3
residual activity [%]	EG	100	92	85
	PG	100	103	102
	PEG	100	102	98

10 <sup>\*)</sup>: EG = ethylene glycol, PG = propylene glycol,  
PEG = polyethylene glycol 400

All publications (including patents and patent applications) mentioned in this specification are indicative to  
15 the level of skill of those skilled in the art to which this invention pertains. All publications are herein incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference.

20 Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope  
25 of the appended claims.

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Claims

1. A granule where the core consists of a porous material in which (part of) the enzyme(s) which is (are) present in the granule is absorbed into the core.  
5
2. A granule where the core consists of a porous material in which (part of) the enzyme(s) which is (are) present in the granule is absorbed into the core and  
10 which is coated with a protective outer layer.
3. A granule where the core consists of a porous material in which (part of) the enzyme(s) which is (are) present in the granule is absorbed into the core and  
15 which is coated with several coating layers. These layers can contain e.g. (other) enzymes, stabilizers, colouring agents, layers to obtain controlled release.
4. A process to produce a dust free enzyme granule, where  
20 - the granule consists of a porous material and is brought into contact with an aqueous or non aqueous enzyme solution or slurry so that the enzyme is absorbed into the granule.  
- when necessary the granule is coated with a  
25 protective outer layer or several coating layers in order to obtain the products which are described in claim 2 and 3.
5. A process as under 4, where the granule consists of a  
30 porous material and is brought into contact with an aqueous or non aqueous enzyme solution or slurry so that the enzyme is absorbed into the granule in a mixer.
- 35 6. As the process under 5, where the apparatus is a fluid bed dryer.



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7. A process as under 4, where the granule is coated with a protective outer layer or several coating layers in order to obtain the products are described in claim 2 and 3 where the coating takes place in a mixer.
- 5 8. A process as under 7, where the coating takes place in a fluid bed dryer.
9. A process as under 4, where the whole process is performed in one apparatus.
- 10 10. A process as under 9, where the whole process is performed in a fluid bed dryer.
- 15 11. A coating layer which consists of a (mixture of) modified cellulose(s); where a compound or compound(s) are added to prevent agglomeration and stickiness of the granules during the coating process.
- 20 12. A coating layer as under 11, where the added compound is talc.
13. By applying a coating to the porous material, loaded with enzyme containing aqueous or non-aqueous liquid, the strength of the particles was found to increase considerably.
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# INTERNATIONAL SEARCH REPORT

Int'l Application No  
PCT/EP 94/01642

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 5 C12N11/14 C12N9/98 C11D3/386		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 5 C12N C11D		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP,A,0 430 395 (KINGSTON DIAGNOSTICS, L.P.) 5 June 1991 see page 3, line 41 - line 52 ---	1,4
A	EP,A,0 206 418 (THE PROCTER & GAMBLE COMPANY) 30 October 1986 see the whole document ---	1-10
A	EP,A,0 193 829 (MILES INC.) 10 September 1986 cited in the application see the whole document ---	1-10
A	US,A,5 177 013 (USUI ET AL.) 5 January 1993 --- <div style="text-align: right;">-/--</div>	
<div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.</span> <span><input checked="" type="checkbox"/> Patent family members are listed in annex.</span> </div>		
<div style="display: flex;"> <div style="flex: 1;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="flex: 1;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
2	Date of the actual completion of the international search  <div style="text-align: center;">5 September 1994</div>	Date of mailing of the international search report  <div style="text-align: center;">01 -02- 1995</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+ 31-70) 340-3016		Authorized officer  <div style="text-align: center;">CEDER O.</div>

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 94/01642

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EP,A,0 542 351 (AKZO N.V.) 19 May 1993 cited in the application see page 8, line 42 - line 44 see abstract	1,4
P,A	cited in the application -----	2,3,5-10

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP 94/01642

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See annex.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1 - 10

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/210

LACK OF UNITY OF INVENTION

1. Claims: 1-10      Granule containing an enzyme in its core and method for producing it.
2. Claims: 11-12    Coating on porous carrier to prevent the carriers from sticking together.
3. Claims: 13        Method for increasing the strength of a particle by applying a coating.

**INTERNATIONAL SEARCH REPORT**

information on patent family members

International Application No

**PCT/EP 94/01642**

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0430395	05-06-91	CA-A- 2028968 JP-A- 3168083	25-05-91 19-07-91
EP-A-0206418	30-12-86	AU-B- 585031 AU-A- 5932286 DE-A- 3682443 JP-A- 62079296 US-A- 4767557	08-06-89 08-01-87 19-12-91 11-04-87 30-08-88
EP-A-0193829	10-09-86	US-A- 4689297 CA-A- 1267622 DE-A- 3681468 JP-B- 4006351 JP-A- 61209589 JP-A- 6062857	25-08-87 10-04-90 24-10-91 05-02-92 17-09-86 08-03-94
US-A-5177013	05-01-93	JP-A- 3130079	03-06-91
EP-A-0542351	19-05-93	JP-A- 5309255 US-A- 5348695	22-11-93 20-09-94